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An old wives' tale. Reproductive outcomes in pregnant women aged 35 or older: the role of individual repair capacity

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Abstract

At present, childbirth is being progressively postponed until later age. Women aged 35 or older may have to wait longer to conceive than younger women and are more likely to be referred to fertility evaluations, but in a significant proportion a spontaneous conception would be achieved in the timeframe typical of younger women. Pregnancies where mothers are ≥ 35 are associated with more risks for pregnancy loss, chromosomal disease, pregnancy-associated complications, prematurity and low birthweight. These concerns, however, are not uncommon in younger women as well. This puts forward the question whether advanced age per se is the underlying cause for the increased risk for adverse outcomes in older pregnant women, or whether there might be other factors that account for it but do not radically worsen the prospects for favourable outcomes. The individual risks associated with childbirth late in life may stem from maternal genetic background rather than being a simple function of age. There is plenty of preliminary evidence that individual capacity for identification and repair of DNA damage may constitute a major factor in female fertility and fecundity. Subtle deficiencies in the repair capacity may have little to no importance in younger pregnant women but may make a significant difference in older women. The outcomes of pregnancies in women >35 are largely dependent on the pre-pregnancy health status and the quality of antenatal care, and may not be dramatically different from outcomes in younger women.

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Abbreviations: 8-OH-dG – 8-hydroxydeoxyguanosine, **AMA** – advanced maternal age, **AR** – assisted reproduction, **BER** – base excision repair, **CDC** – US Centre for disease prevention and control, **DIC** – disseminated intravascular coagulation, **DS** – Down syndrome, **HELLP** – haemolysis/elevated liver enzymes/low platelets, **ICSI** – intracytoplasmic sperm injection, **IRC** – individual repair capacity, **IUGR** – intrauterine growth restriction, **IUI** – intrauterine insemination, **IVF** – *in vitro* fertilisation, **LIF** – leukaemia inhibitory factor, **NER** – nucleotide excision repair, **PCOS** – polycystic ovary syndrome, **PIH** – pregnancy-induced hypertension, **ROS** – reactive oxygen species, **TTD** – trichothiodystrophy, **w.g.** – weeks of gestation, **WHO** – World Health Organisation, **XP** – xeroderma pigmentosum.

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1. Fertility issues in couples where the woman is 35 years of age or older - now we see them, now we don't

According to the definition of the International Federation of Gynaecology and Obstetrics (FIGO) in 1958 on "Elderly primigravidae", advanced maternal age (AMA) was defined as 35 years and older at the time of birth of the child. In the last 25 years, the age of birth of first child has shifted significantly towards later reproductive years. Among the developed countries, the age of first birth in 2015 has been lowest in the US (26 years, according to the US Centre for disease prevention and control (CDC). At the same time, 20 % of the women in the US have their first child after the age of 35 [1]. In Europe and in developed Asian countries, the age of first birth is even higher. The average age at first birth in Japan in 2013 was 30.4 years [2]. In 2013, the age of first birth in EU countries was 28.7, with Bulgaria and Romania being at the lower end of the scale (first-time mothers giving birth at the age of 25.7 and 25.8, respectively) and Switzerland, Spain and Italy being at the other extreme, with age of birth of first child at 30.4, 30.4 and 30.6 years, respectively [3]. The tendency of delaying childbirth (and, specifically, first birth) until later age has been stable in Europe for the last 15 years.

About 80 % of the couples that are trying to have a child achieve conception within 6 months of unprotected intercourse. After a total of 12 months, the rates of natural conception rise to 85-90 % of all couples [4]. This is the basis of the definition of infertility adopted by the World Health Organisation (WHO): "*a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse*." [5]. Nevertheless, half of the couples that fulfil the WHO criterion for infertility (another 5-8 %) are likely to conceive without assistance in the following 24 months and only the remaining 2-5 % may be in need of assisted reproduction (AR) techniques.

Identification of a physical cause for the couple's infertility is usually possible in about 85-90 % of in couples with reproductive issues. The contribution of female and male infertility is roughly equal. About 40 % of infertile couples have a combination of male and female factor infertility [6]. The age of the woman in a couple trying to conceive is considered to be one of the most significant factors determining the chances for conception (natural or using assisted reproduction (AR) techniques) as well as the chances for successful pregnancy outcome.

An estimated 30-40 % of the couples in which the woman is older than 35 years may face fertility issues [7]. Thus, in couples where the AMA is a concern, the period of waiting to conceive before a fertility evaluation is recommended is usually shortened to 6 months. The latter takes into account that the evaluations of the cause for infertility and the potential interventions in order to achieve successful pregnancy may take significant amount of time, in the order of months and years.

The shorter period of expectant management and the lower rates of conception within 6 months mean that older women are more likely to be referred to fertility evaluations and to be offerred AR techniques in order to achieve a pregnancy. While fertility evaluations and subsequent interventions are undoubtedly important for the successful resolution of fertility issues, it would do well to remember that in couples where the woman is ≥ 35 the spontaneous pregnancy rates usually reach 85 % within 48 months. Therefore, the absolute pregnancy rates in these couples may not be very different from the 48-months rates observed in younger couples.

2. Increased risks for adverse outcomes in pregnancies after age 35 - is age really the cause?

The age of the woman in the couple is believed to play a role not only in the chance for conception (fertility), but also in the capacity to sustain pregnancy and carry successfully to term (fecundity). Generally, the risks for pregnancy loss, pregnancy complications or adverse outcomes at different stages increase with maternal age.

The risk for spontaneous loss of pregnancy is unusually high in humans.

In young women without recognisable risk factors, an estimated 40-50 % of early embryos are lost around the time of implantation, that is, before the pregnancy could be clinically recognised [8]. 13-15 % of the clinically recognised pregnancies are subsequently lost in the first 10 weeks of gestation (w.g.). The risk for early pregnancy loss increases in older women. At the age of 35, about 20 % of clinically recognised pregnancies may be expected to be lost early in pregnancy. At the age of 42, pregnancy loss in the first 10 weeks occurs in approximately half of the pregnancies (55%). By the age of 48, the early pregnancy loss rate may be as high as 84 % [9]. Generally, early pregnancy losses are related to severe defects in embryonic development, including chromosomal disorders. The risk for trisomies 21, 13 and 18 and sex chromosome aneuploidies are known to increase with advancing maternal age. The increase becomes significant after the age of 30, contrary to the popular belief that only women ≥ 35 are at risk for having a child with chromosomal defects. The estimated rate of all clinically significant cytogenetic abnormalities is about 1/500 in women aged 25-29, 1/ 270 at age 30, 1/80 at the age of 35, 1/60 at the age of 40 and 1/20 at the age of 45 [10, 11]. The age-related risk for the most prevalent chromosomal disorder (Down syndrome, DS)

is estimated to be about 1/1 250 for women aged 25-30 years of age. At the age of 35, the risk for DS increases to about 1/300 [10]. Since this risk is close to the value of the risk for pregnancy loss after invasive prenatal diagnostics by amniocentesis (1:200 or lower, depending on the quality of the imaging equipment and the experience of the operator). Thus, the age of 35 and/or a risk determined by biochemical screening at 1:250 or higher estimated by different screening modalities have been selected as cutoff values beyond which invasive prenatal diagnosis is usually recommended [11-13]. At the age of 40 and 45 the risk for having a child with DS increases to 1/100 and 1/30, respectively.

Nevertheless, about 70-80 % of the children with Down syndrome are born to women younger than the age of 35. This is primarily related to the fact that the majority of women pregnant at any given time are < 35. Despite the fact that the percentage of pregnant women ≥ 35 has been growing for the past 10-20 years, a significant increase in the incidence rates of clinically significant chromosomal defects has not been noted yet. This may be explained with the high sensitivity and specificity of the screening methods that are currently in use and the and the accessibility and financial affordability of the screening tests.

There are biological mechanisms that increase the risk for conceiving a child with chromosomal defects at advanced maternal age. This risk, in itself, is unavoidable. At the same time, giving birth to a child affected with chromosomal disease or neural tube defect is preventable.

The incidence of ectopic pregnancy increases with AMA. The rates of ectopic pregnancy in women aged 20-25 is about 1.5 - 2 %, whereas in women aged 44 years and older these are reported to be about 7 % (3-4-fold increase) [9, 14]. This may be related to age-dependent effects, but also to the fact that older women are more likely to have a history for previous ectopic pregnancies, pelvic inflammatory disease, infections (gonorrhoea, chlamydiasis and others), history of pelvic surgery, use of intrauterine devices and smoking, all of these being risk factors for ectopic pregnancy.

Complications of pregnancy are significantly more common in pregnant women ≥ 35 and may be associated with higher risks for fetal demise. These risks, however, may be related to pre-existing diseases and conditions that became exacerbated during pregnancy. For example, the risk for developing preeclampsia is 2- to 3-fold elevated for pregnant women aged ≥ 35 compared to women aged 25-29 [15]. Older pregnant women are more likely to be obese and to have pre-existing hypertension, hyperglycemia and dyslipidemia, all of these being known risk factors for development of preeclampsia. Women with hypertension during pregnancy (either pre-existing or gestation-induced) may be more susceptible to specific antepartum and intrapartum complications such as placental abruption, ischemic stroke and disseminated intravascular coagulation (DIC) syndrome. Foetuses of these mothers are at greater risk of intrauterine growth restriction (IUGR), preterm delivery and intrauterine death [16-18]. Older women are more likely to give birth to babies with low birthweight (< 2500 grams) or high birthweight (>4 000 grams), both of which constitute risk factors complicated delivery. Placentation issues (placenta previa, vasa previa, placenta accreta and the less common variants of placenta increta and placenta percreta) are more frequent in pregnant women ≥ 35 compared to younger women [19, 20]. This may or may not be related to the fact that older women are more likely to have previous pregnancies and a history of uterine surgery, both discussed as probable risk factors for abnormal placentation. Placental implantation abnormalities are associated with increased risk for preterm delivery and the consequently increased perinatal morbidity and mortality.

Advanced maternal age is believed to be associated with increased rates of stillbirth. The absolute risk of stillbirth for women 25-29 years old is about 1:1 000 ongoing pregnancies, whereas the risk for women aged 35-39 years old and women ≥ 40 years old is estimated at 1:382 and 1:267 pregnancies, respectively [21]. The study of Bahtiyar et al. reported that the risk for intrauterine fetal demise at 39 gestational weeks for women 40- 44 years of age was comparable to the risk in women 25 to 29 years of age at 42 weeks (which is usually twice the risk at 39-40 weeks) [22]. There are other authors that report that the increase of the risk for stillbirth after the age of 35 was less pronounced than the corresponding increase of the risk for spontaneous pregnancy losses and ectopic pregnancies [9].

Increased rates (over 4-fold) of spontaneous multiple pregnancies, specifically with dizygotic twins are observed in older mothers [16, 23]. The causes for this phenomenon have not been completely identified, although several hypotheses have been proposed, including the hypothesis for the 'terminal reproductive effort" - a reproductive strategy based on significant investment in the reproductive events at the end of reproductive age [24]. Pregnancy with multiples is considered a risk factor for adverse outcomes even in younger women, as it is associated with increased risk for preterm delivery, low birthweight, placental abruption, gestation-induced hypertensive disorders and gestational diabetes. Interestingly, in primiparous women, the perinatal outcomes of twin pregnancies (regardless of the type of conception - spontaneous or using AR techniques; the socioeconomic status of the mother and twin zygocity) have been repeatedly reported to be better for women ≥ 35 than for women aged 25-29 [25,26]. The favourable outcomes of multiple pregnancy in older women are an yet unresolved paradox, considering that

the perinatal outcomes of singleton pregnancies are generally worse in older women than in younger women. A recent study showed that the causes for the improved outcomes of multiple pregnancies compared to singleton pregnancies in women aged ≥ 35 may have their basis in the interaction between mechanisms that modulate the risks for pregnancy complications. In other words, is possible that the pathogenetic mechanisms of some of the diseases and conditions that are statistically to be expected to develop at higher rates in older gravidas may be so intricately interlinked that they may influence each other in a complex manner that may be associated with increased risks for an adverse outcome but also with increased chances for a positive outcome, depending on the context in which they develop. Specifically, a recent study in a large group of pregnant women showed that having placenta previa (but not placenta accreta) was associated with a significant reduction in the incidence of pregnancy-induced hypertension (55 %) and preeclampsia (86 %) [27]. As placenta previa is more common in older pregnant women and in multiple pregnancies (which are also more common in older women), it is possible that the effect of one condition with a potential for adverse outcomes (but still manageable, as is placenta previa) may actually result in reduction of the risk of other serious complications of pregnancy such as pregnancy-induced hypertension (PIH) and preeclampsia.

Advanced maternal age is a predisposing factor for virtually all complications of pregnancy. Nevertheless, an older gravida may have just as favourable an outcome as a younger woman, provided that adequate antenatal care (based upon a combined strategy of informed decision-making and early diagnosis and intervention) is available. At present, it is possible for women of advanced maternal age to have successful pregnancies with favourable outcome rates not significantly different from those of younger women if pre-existing chronic diseases and pregnancy-induced diseases conditions were timely recognised and adequately treated [17, 20, 28].

The risks for age-related chromosomal abnormalities in the foetus may be an object of special concern in the first and second trimester, whereas late complications of pregnancy (preeclampsia, uterine bleeding due to placenta previa/vasa previa or placental abruption), IUGR and risks for preterm delivery may dominate the third trimester. These concerns, nevertheless, are not uncommon in younger women as well. Logically, a question arises whether advanced age as such (and the accompanying diseases and conditions) may be the underlying cause for the increased risk for adverse outcomes in older pregnant women, or whether there might be other factor/s that may account for the age-related increase of the risks but do not radically worsen the prospects for favourable pregnancy outcomes for women ≥ 35. Below we will speculate on the nature of such factor/s, their potential effect on pre-conception, conception/implantation and in the course of pregnancy and the age-dependent effects that may modulate their impact in older gravidas.

3. Individual capacity for repair of genotoxic damage - invisible in peace, (almost) invincible in war

Biomolecules in living cells are subjected daily to significant amounts of damage. The majority of the damaged molecules are replaced shortly after the damage has occurred in order to prevent lasting effects on the integrity of the cell. Only one type of biological molecule - namely, genomic DNA - cannot be replaced as it represents the blueprint for synthesis of the major biological polymers in the living cell, including its own synthesis. Damage to the cellular genome is, instead, repaired on a regular basis. The efficiency of the cellular repair mechanisms is normally very high. The average eukaryotic cell has to manage no less than 10^4 - 10^5 instances of damage per day [29]. At the same time, the mutation rate of the mammalian nuclear genome is only about 5×10^{-9}–1×10^{-8} per base per generation [30]. Thus, DNA damage is normally promptly managed in young clinically healthy individuals.

Until several decades ago, it was believed that decreased capacity to repair genomic damage unavoidably resulted in severe disease. Indeed, the first human disease that was linked to deficiency in DNA repair (xeroderma pigmentosum, XP) was characterised by an early-onset severe phenotype that usually caused death in childhood or adolescence [31]. The same was valid for the other two major 'diseases of repair' - Cockayne syndrome and trichothiodystrophy (TTD). A decade later, nevertheless, it was reported that there was significant variance in the efficiency of repair of DNA damage between batches of cultured lymphocytes isolated from different clinically healthy human volunteers [32]. What is more, lymphocytes isolated from women's blood were more efficient in repairing genotoxic damage than lymphocytes isolated from men's blood. Several years later it was reported that susceptibility to cancer following exposure to known carcinogens also exhibited inter-individual variance and gender-dependent variation [33]. Apparently, deviations from the 'average' level of efficiency of repair of DNA damage were not always associated with severe disease and there were genetic factors determining the capacity to repair DNA damage. Among the first of these genetic factors to be identified was the common Pro72Arg polymorphism in the *TP53* gene (rs1042522), a C-to-G transversion in exon 4 of the *TP53* gene [34]. Later, it was unequivocally confirmed that there was genetically predetermined individual variation in the capacity to

repair DNA (individual repair capacity, IRC).

Carriership of genetic variants conferring DNA repair capacity different (subtly lower or, in rare cases, subtly higher) than the average is not associated with immediate negative or positive effects, at least not until the carrier individuals are young and (generally) healthy. As the individual ages, however, the capacity to recognise and repair DNA damage gradually declines. Thus, individual variance in IRC may become more pronounced and/or may determine the susceptibility to common diseases and conditions in later age [35, 36], the outcomes of therapies (especially those based on genotoxic effects such as anticancer and immunosuppressive therapies) and the risk for development of therapy-associated adverse effects [36, 37]. IRC is a key determinant of the capacity for cell and tissue renewal and plays a major role in the risk for development of cancer and degenerative disease [37-40]. Studies of individual repair capacity are currently part of the rapidly expanding field of individualised (personalised) medicine, dedicated to tailoring of therapies to meet the needs of a particular patient, assessment of eligibility for specific types of therapy, prognostication of outcomes from different therapies and anticipation of potential adverse effects.

At present, the role of IRC in mammalian (specifically, human) fertility is under intensive study. The association of carriership of variant alleles of genes coding for major proteins of DNA repair with fertility and risks for early pregnancy loss has already been demonstrated [41-45]. Some of the associations may have radically opposite effects in the constitution of male and female fertility [41, 45, 46]; may have different strength at different ages (carriership of a specific variant allele may not confer increased risk for reproductive failure in younger individuals but may become significant in older individuals and vice versa) and may have dissimilar effects in populations of different racial and ethnic origin [43, 47].

In the present paper we propose that the well-known age-related effects on female fertility and the increased risks for adverse pregnancy outcomes in the older gravida may be related not to advanced 'age' as such, but to maternal capacity to detect and repair genotoxic damage and the health risks associated with subtle but lasting deficiencies of repair capacity. IRC apparently plays a role in the repair of damage in the haploid parental genomes in the course of fertilisation, in the maintenance of the viability of the embryo in the early days and weeks of pregnancy and in limiting the inflammatory and apoptotic responses triggered by high levels of unrepaired DNA damage in order to ensure adequate placentation and normal foetal growth. Thus, the individual risks associated with childbirth late in life may result from the complex interaction between the maternal genetic background (establishing the bases of propensity for pre-existing and pregnancy-induced disease) and environmental factors that may trigger and/or promote development of disease. In the near future it may be possible to use the accumulated data about the role of IRC in human fertility and fecundity to appraise the chances for successful pregnancy by natural means or using the opportunities provided by modern AR techniques and to predict reliably the risks for adverse pregnancy outcomes, especially in older pregnant women.

4. Role of individual repair capacity in oogenesis and oocyte maturation

Male and female gametes in mammals (and, in fact, in all higher eukaryotes) are very dissimilar to one another in virtually all aspects - size, morphology and biological properties. The mature male gamete (spermatozoon) is much smaller and significantly more mobile than the female gamete. It is also relatively short-lived, as it is equipped only with the bare basics for its survival and, potentially, fulfilment of its biological function - a large number of mitochondria to provide rapid energy production, a tail with specific cytoskeletal organisation to ensure the capacity for rapid movement, an acrosome containing the hydrolytic enzymes needed for penetration beyond the outer protective layers of the oocyte; and a haploid nucleus with densely packed DNA. Nuclear transactions (replication, transcription, DNA repair) are completely suppressed in the mature spermatozoon. In contrast, the mature female gamete (oocyte) is significantly larger and needs to be passively propelled from the ovary to the uterine cavity. It also contains significant amounts of pre-stocked RNA transcripts, proteins, and energy sources and a full set of cellular organelles, as the first divisions of the prospective zygote are to be provided for by the oocyte's own reserves. Thus, the resources of the male gametes are allocated to traits that enhance success in the competition for fertilisation whereas female gametes allocate their resources primarily to the offspring [48].

The earliest stages of human oogenesis occur during intrauterine life of the female foetus. In the early weeks of the second trimester, multiple mitotic divisions of the precursor cells of the germinal epithelium occur, resulting in 6-7 millions of diploid oogonia in the developing foetal ovary. In 18-22 w.g., a mass apoptotic wave decreases the number of oogonia (oogonial atresia), limiting the number of potential primordial follicles to 4-5 million [49]. At this point, the foetal ovary contains its peak number of cells that may, potentially, give rise to oocytes in later life. Around week 20, the first meiotic division begins and proceeds up to the diplotene stage of prophase I (primary oocyte), where it is arrested until the onset of

puberty. By weeks 22-24, a second wave of apoptotic death (follicular atresia) occurs that decreases further the number of potential oocytes in the female foetus, up to about one million at the moment of birth on the average, although the individual numbers may significantly vary - from about 25 000 to 1.5 million [50, 51]. Thus, the intrauterine period of oocyte maturation is characterised by two major rounds of selection via apoptosis - one at oogonial (pre-meiotic) stage, and one at follicular stage. Follicular atresia continues in individual life, reducing the number of potential follicles to an average of 300 000 at puberty (although the numbers vary), about 10-15 % of this initial number at age 30 and about 3 % at age 40 [51]. The rate of follicular atresia increases with age, so that at the age of 50, only about 1 000 potential follicles remain, regardless of the initial numbers of primordial follicles. Nevertheless, as women may have very different numbers of primary oocytes after the first round of apoptosis (weeks 18-22), and, respectively, a very different number of primordial follicles at birth, the above mentioned percentages of follicles remaining after the age of 30 may, in some women, be comparable to the amounts present in the ovaries of women significantly younger or to the very few follicles remaining in the ovary at the age of ≥50. This theory was first proposed by Wallace & Kelsey in 2010 [51] and still has not been confirmed unequivocally, partly because of ethical considerations regarding studies conducted in human subjects, partly because of the relatively new theory of the possibility of oogenesis occurring at postnatal stages, albeit at a very low rate [52]. The fact is, nevertheless, that, there are women aged ≥ 35 with ovarian reserve close to that of younger women and, respectively, similar natural six-month conception rates. At the same time, there are women with initially poorer ovarian reserve that declines much more rapidly than expected for their age and general health status. A relatively recently published study demonstrated a definite link between a woman's ovarian reserve (measured by anthral follicle count and serum levels of anti-Müllerian hormone) and her mother's age at menopause as a retrospective indirect measure of ovarian reserve in later years [53]. These results indicate that there may be heritable component/s in the constitution of female fertility. Thus, it may not be age per se that determines the capacity to produce oocytes fit for fertilisation (except, of course, in advanced age), but, rather, another factor, that is age-dependent, but is still subject to significant individual variation.

As was already mentioned, potential human oocytes are subjected to serial selections from the stage of oogonia onwards. The selection proceeds in the manner typical of differentiating cells, using criteria of the type 'fulfils requirements in order to proceed to the next stage' and 'has received a survival signal', with all cells that do not comply with these criteria being routed to the programmed cell death pathway. DNA replication is significant source of potential DNA damage, not only because unpacked and untangled DNA is vulnerable to genotoxic insults but also because of the inherent error-proneness of DNA synthesis. Cell division is controlled by strict checkpoints that dictate that all DNA must be replicated and that only very low levels of unrepaired DNA damage (ideally-none at all) must be present in order to enable the cell to proceed before actual division of cell components. Therefore, checks for integrity of DNA and the presence of errors are carried out in at least three crucially important checkpoints during different phases of the cell cycle: the G1/S phase checkpoint; the intra-S-phase checkpoint and the G2/M phase checkpoint [54]. The G1/S checkpoint is the most stringent of these checkpoints and is tightly linked to the system of DNA repair and/or initiation of apoptosis. In cells of early human embryos the stringency of the G1/S checkpoint is somewhat relaxed (in order to allow for rapid division) but it is still in place [55]. The amount of residual unrepaired damage in rapidly dividing cells depends, very broadly, on the number of divisions, the efficiency of checks for DNA damage before every division and the efficiency of DNA repair. In the presence of unrepaired DNA damage eukaryotic cells generally have to choose between three possible pathways: cell cycle arrest and repair of damage; initiation of apoptosis in order to eliminate damaged cells; or, in special cases only, an alternative mechanism that allows replication of damaged templates (error-prone template copying). The decision whether to use any of these pathways depends on multiple factors, endogenous as well as exogenous, and is made specifically for every particular case. In the course of cell differentiation, the first and the last of these three possibilities are not viable options. Instigation of cell cycle arrest in dividing oogonia in order to allow additional time for DNA repair is not compatible with the very tight timeframe of the development of the early human embryo and error-prone copying may result in rapid accumulation of errors in DNA. Apoptosis is the only possible outcome for oogonial cells that have not been deemed 'fit' to proceed to first meiotic division because of cell cycle arrest imposed by the cellular checkpoints upon detection of DNA damage beyond a certain (very low) threshold value. Thus, despite the fact that the starting number of diploid oogonia is more or less similar in female foetuses, the number of potential oocyte precursors after the first rounds of apoptotic selection may be very different in different female foetuses. Considering that DNA damage is one of the most potent triggers of apoptosis, the factor that determines the number of pre-meiotic cells that have

escaped apoptosis by week 18-22 of gestation may well be the efficiency of detection and management of genomic damage.

Individual capacity for DNA repair may play a role in follicular atresia as well. Apoptotic death of follicles is mediated primarily by apoptosis of granulosa cells, the hormone and growth factor-secreting cells supporting and stimulating the development of the oocyte. It is generally believed that the main factor determining the outcomes of the apoptotic selection for different follicles is whether they have received adequate gonadotropin support [49]. In 1999, it was demonstrated that cell cycle arrest occurring at the G1/S transition checkpoint because of high level of unrepaired DNA damage was sufficient to force the follicle into atresia via up-regulation of the endogenous (p53-dependent) and exogenous (receptor-mediated) death pathways in granulosa cells [56]. Thus, the efficiency of DNA error detection and repair may play a role in follicular escape from atresia, at prenatal as well as at postnatal level, with levels of DNA repair close to normal possibly determining a higher number of non-atretic follicles at birth, slower rates of follicle attrition and, respectively, better ovarian reserve at later age.

Quiescent follicles do not replicate their DNA any further (a pre-requisite to generation of a haploid genome) and normally have a very low metabolic rate. The latter means that they generate very low levels of radical oxygen species (ROS) with a potential to damage the DNA of the oocyte or the surrounding cells. There is also the fact that genetic recombination (occurring in prophase I) is associated with legitimate generation of multiple instances of reactive DNA ends. Presence of free DNA ends is one of the most potent triggers for recruitment of DNA repair machinery; therefore, maintenance of near-normal DNA repair capacity in cells undergoing meiotic recombination may cause unneeded activation of the repair mechanisms. Thus, the capacity for DNA repair is preserved in the quiescent oocyte, albeit at a baseline level only. As the oocyte quiescence in humans lasts for decades, it increases the risk for occurrence of random DNA damage. This minimal damage is normally promptly repaired and subtle variations in the repair capacity matter very little in the quiescent mammalian oocyte. Of course, the prolonged arrest in prophase I may increase the risk for random association and subsequent ligation of free ends, increasing the risk for chromosomal fusion and breakage (indeed, nondisjunction of the two 21 chromosomes at prophase I is believed to be the cause of at least two-thirds of the Down syndrome cases [57]. DNA repair becomes upregulated (over 15-fold) in mammalian follicles after stimulation towards growth compared to quiescent follicles [58]. The damage profile in follicles stimulated towards maturation may be very different from the profile of quiescent follicles, as the active metabolism in the growing follicle means higher rates of generation of ROS, associated with increased levels of oxidative damage in DNA (oxidised bases, strand breakage) and there is increased risk for replication errors occurring in actively dividing granulosa cells. It is possible that exactly at this stage the individual variance in the capacity for DNA repair may play a role in modulation of follicle atresia. Thus, quiescent follicles with sub-optimal DNA repair may manage DNA well enough, but may not be able to cope with the increased levels of damage occurring in the course of follicle growth and maturation. It could be expected that subtle deficiencies in DNA repair (conferred by co-carriership of a set of variant alleles) may increase the rates of follicular atresia and, respectively, increase the risk for diminished ovarian reserve at later age. The studies in the field are still very few, but intriguing patterns have already started to emerge.

Mitochondrial DNA generally bears the brunt of oxidative damage in living cells, partly because of its physical proximity to the main source of oxidative stress (the electron transport chains in the inner mitochondrial membrane) and the specific mode of histone-free packaging. There is very little polymorphism in mitochondrial DNA because of the increased gene density, but once a benign variant occurs; it may be transmitted down many generations via the female line. Since mitochondrial DNA is virtually never subject of recombination, a set of mitochondrial polymorphisms on the same molecule usually segregates as a single unit (mitochondrial haplogroup). Single polymorphisms that make up a haplogroup have very little effect on the general properties of carrier mitochondria, but some mitochondrial haplogroups as a whole may be associated with differential oxidative capacity. Specifically, the haplogroup H (the most common mitochondrial haplogroup in Europe) is associated with higher-than-average oxygen consumption and ATP output whereas the related haplogroups J and T are associated with lower-than-average oxygen consumption and ATP output [59]. The levels of production of ROS are therefore highest for haplogroup H and lowest for haplogroups J and T, respectively. Recently, it was reported that the risk for diminished ovarian reserve was 3-fold reduced in female carriers of haplogroups J and T than in age-matched carriers of any other mitochondrial haplogroup [60]. Notably, haplogroup J has been shown to be also overrepresented in healthy individuals with extreme longevity, including healthy centenarians [61, 62]. The latter may give some clue as to a potential cause of another phenomenon, namely, the fact that increased age of birth of last child may be associated with increased female longevity [63, 64]. The authors of the latter two

studies specifically point out that the relationship seen between late childbirth and longevity may simply be an expression of generally slower ageing pattern. Thus, the increased prevalence of carriership of a mitochondrial DNA of haplogroup J both in long-lived individuals and in women with preserved ovarian capacity until later age may not be a coincidence, but, rather, a result of decreased levels of oxidative stress allowing for more efficient management of genotoxic damage and, respectively, for delayed effects of physiological ageing. At present, there is very little data about the role of IRC in the establishment and maintenance of ovarian reserve, but it is possible that the rapid decline of ovarian reserve seen in some women (and which, according to [53] is least partly hereditary) may be related to carriership of genotypes combining a decreased efficiency for oxygen utilisation (conferred by specific mitochondrial haplogroup, such as H) and a heritable subclinical deficiency in the capacity for detection and repair of DNA damage. The two factors are likely to act together on a prenatal level (resulting in reduced number of primordial follicles/reduced number of quiescent oocytes) as well as on postnatal level (increased rates of follicular attrition).

The capacity for DNA repair of the oocyte has another very important function (apart from the maintenance of the integrity of its own genome) that becomes apparent at the stage of fertilisation. It was already mentioned that mature spermatozoa are virtually incapable of repairing their own DNA, as it is very tightly packed and the proteins and organelles of the DNA repair machinery are lost in the course of differentiation. The mature sperm needs a lot of energy provided by numerous mitochondria located in its midpiece. The intensive oxidative metabolism of the spermatozoon generates a significant amount of ROS with genotoxic potential. The packaging of the genome of the sperm decreases the risk for occurrence of damage, but once it occurs, it cannot be repaired. Thus, the quality of sperm DNA is characterised by its level of fragmentation. It has long been noted that the level of fragmentation negatively correlates with the success rates of in vitro fertilisation (IVF)/ intracytoplasmic sperm injection (ICS) and embryo cleavage [65]. Nevertheless, if a spermatozoon manages to successfully reach and penetrate the mature ovum, the intact repair system of the oocyte normally takes over and is capable of restoring the integrity of the paternal genome, provided that the levels of fragmentation do not exceed a certain threshold. For intrauterine insemination (IUI), the maximum tolerated level of sperm fragmentation that could result in successful pregnancy was estimated to be significant, about 12 % [66]. Apparently, near-normal or superior maternal capacity for DNA repair may also play a role at early post-fertilisation stages by partially compensating for defective spermatogenesis in the male partner.

5. Role of individual repair capacity in the constitution of the risk for diseases and conditions that may decrease the chances for conception

Carriership of several common polymorphisms in genes coding for key proteins of DNA damage detection and repair and/or maintenance of genomic integrity has been associated with modulation of the risk for certain diseases and conditions that affect female fertility, e.g. endometriosis and polycystic ovary syndrome (PCOS). A prime example is the already mentioned Pro72Arg polymorphism in the *TP53* gene [34]. *TP53* codes for p53, a DNA-binding protein with key roles in the regulation of the progression through the cell cycle and apoptosis. Both allelic variants are considered wildtype, as they exhibit no significant differences with respect to p53 conformation and DNA-binding affinities. Nevertheless, the variant alleles have differential biological properties. Specifically, the *TP53* 72Pro variant is a stronger transcription activator (specifically, activator of transcription of genes coding for products implicated in induction of cell cycle arrest in the presence of DNA damage and DNA repair) than 72Arg), whereas *TP53* 72Arg is a more potent inductor of apoptosis than the 72Pro allele [67, 68]. It has repeatedly been shown that the carriership of the 'repair-prone' 72Pro allele and especially of the homozygous Pro/Pro genotype was significantly overrepresented in women with endometriosis than in controls [42, 45, 69]. The prevalence of endometriosis peaks in the age group between 35 and 44, reaching 1-1.5 % [70]. Moderate-to-severe endometriosis (stages II-IV) is associated with decreased chances to conceive naturally. The outcomes after IVF/ICSI in women with endometriosis of all stages are significantly poorer in terms of dosage and duration of gonadotropin treatment, number of oocytes retrieved, fertilisation rate, number of obtained embryos, and number of quality embryos of day 3 post-fertilisation [71]. It has been speculated that the 'pro-repair' tendencies conferred by carriership of the 72Pro allele may increase the risk for ectopic growth of endometrial tissue [72]. Female carriers of the variant (duplication) allele of another common *TP53* gene polymorphism - the 16 bp duplication polymorphism were also found to be at significantly increased risk for endometriosis [45]. Similarly to the 72Pro allele, the 16 bp duplication allele confers slightly increased propensity for cell proliferation [73].

Carriership of polymorphisms in genes coding for regulators of p53 may also have an effect on female fertility. A recent study showed that a polymorphism in the gene coding for the major negative regulator of the stability and activity of p53 - the E3 ubiquitin ligase MDM2 - was associated with modulation of the risk for polycystic ovary syndrome (PCOS) [74]. PCOS is one

of the most common endocrine abnormalities in women of reproductive age and may be a serious risk factor for infertility, as it may interfere with ovulation patterns or altogether preclude ovulation. The variant (G) allele of the *MDM2* SNP309 polymorphism (rs2279744) is known to be associated with higher levels of MDM2, resulting, in turn, in lower levels of p53 and, respectively, decreased p53-dependent suppression of cell proliferation [75], which may explain its association with increased risk for endometrial cancer [76]. The association with increased risk for PCOS was identified for the wildtype (T) *MDM2* allele [74]. PCOS is characterised by follicular growth arrest at early antral stage and low levels of proliferation of granulosa cell proliferation, therefore, a genetically determined propensity for increased cell proliferation may decrease the risk for PCOS. The risk for PCOS has also recently been found to be modulated by carriership of the variant allele of another common polymorphism, namely, the C677T polymorphism in the *MTFHR* gene [77]. The latter codes for 5,10- methylene tetrahydrofolate reductase, a key enzyme in the folate metabolism. The variant T allele is associated with lower enzymatic activity of the resultant protein [78]. Carriership of at least one T allele significantly increases the risk for PCOS [77]. Interestingly, the effect exhibited ethnic variance, as the risk was significantly increased (over two-fold) in women of Caucasian ethnic origin, whereas in women of Asian ethnic origin the increase of the risk for carriers of the T allele to present with PCOS amounted only to 29 %.

The *TP53* Pro72Arg allele and other polymorphic alleles of genes with roles in DNA repair and maintenance of genomic integrity were shown to play a role in the constitution of the chance for twin pregnancies. Specifically, carriership of the Pro72 allele and the rs1563828 polymorphism in a gene closely related to *MDM2* - *MDM4* were shown to be associated with increased rates of twinning, both monozygotic and dizygotic, with the odds ratio for carriers of Pro72 allele to have twin pregnancies being elevated almost 3-fold [79]. This may account, at least partly, for the increased rates of twinning observed in pregnancies in older women, as since the Pro72 allele is associated with decreased female fertility, the 72Pro carrier females are likely to achieve their first successful pregnancy at later age than noncarriers.

6. Role of individual repair capacity for the chances of natural or assisted conception

Female carriership of the 72Pro allele of the *TP53* Pro72Arg polymorphism has been associated with decreased chances for conception [43, 44, 47]. The may be modulated by age. In the study of Corbo et al. [47] that examined the relationship between the *TP53* Pro72Arg genotype, maternal age at birth of children and number of children per family couple as a measure of reproductive efficiency, in women having their first children at 25-29 years of age, carriership of the 72Pro allele was not found to be associated with poorer reproductive efficiency, whereas in those aged 30 or more at birth of fist child, carriership of the 72Pro allele and especially the homozygous Pro/Pro genotype was associated with a 2.67-fold reduction in reproductive efficiency. Apparently, the effects of the 72Pro allele on the reproductive capacity only become significant at later age. There is also the fact that there are racial and ethnic disparities in the ovarian reserve, onset of menopause and outcomes after IVF/ICSI. Specifically, women of Caucasian origin were repeatedly reported to exhibit superior indices of ovarian reserve (levels of AMH, anthral follicle count), later onset of puberty and later onset of menopause and better outcomes after IVF than age-matched women of African, African-American, Hispanic and South Asian origin [80-83]. This phenomenon may be at least partly linked to the specificities in the geographic distribution of the allelic frequencies of the two alleles of the *TP53* Pro72Arg polymorphism. It is very common in all populations, but there is pronounced clinal variation of the allele frequencies. The ancestral 72Pro allele is significantly more common (up to 70 %) in areas close to the equator and in individuals of African ethnic origin [84]. The Arg allele is significantly more common (up to 80 %) in the far North and in individuals with Caucasian ethnic origin. It is believed that the Arg allele has arisen in the course of migration of human populations from tropical to temperate zones as a mechanism for protection of lighter human skin against the harmful effects of UV radiation while, at the same time, preserving the capacity to synthesise enough vitamin D3 to keep the organism healthy [84, 85]. Thus, the increased frequency of the 72Pro allele in individuals with ethnic origin other than Caucasian may account for their adverse fertility profile at later age. The fertility-decreasing effects of the 72Pro allele may have not been actively selected against in the course of human evolution. On the one hand, carriership of the 72Pro allele has a positive effect on male fertility, at least in some populations [86], which may partially compensate for its adverse effects in female fertility. On the other hand, in the course of the emergence of the 72Arg allele (in the time period between the human migration out of Africa (125 000 years ago) and into Europe (40-35 000 years ago), if the adverse effects on fertility conferred by the 72Pro allele only become significant after the age of 35, they mattered very little, as the average human lifespan is likely to have been shorter than 35.

There are other authors that report that in their study groups the effects of carriership of polymorphisms associated with female infertility were more pronounced in younger (>35 years) women than in older women [43]. The authors of the latter study, however, warn that this effect may be due to the increased rates of other type of issues in older women trying to conceive, such as chromosomal defects leading to very early embryonic death and chronic diseases decreasing the chances for pregnancy (obesity, diabetes, hypertension) that had more relative weight as a factor for decreased fertility than the effects conferred by the 72Pro allele.

The effects of carriership of variant alleles of *TP53* may be significant in embryo implantation and at post-implantation stages as well. Carriership of the *TP53* 72Pro allele is overrepresented among women with idiopathic recurrent miscarriage [41, 87] and in women with recurrent implantation failure after IVF and embryo transfer [45, 88]. In the latter study, the pregnancy rates after IVF were reported to be close to 70 % for women with genotypes containing at least one 72Arg allele (Arg/Arg or Pro/Arg) vs. 33 % for women with homozygous Pro/Pro genotypes; the embryo implantation rates were 33 % for women with 72Arg-containing genotypes vs. 7 % for women with Pro/Pro genotypes; and ongoing pregnancy rates were 53 % for women with 72Arg-containing genotypes vs. 14 % for women with Pro/Pro genotypes. The effects of p53 on implantation are believed to be exerted primarily via leukaemia inhibitory factor (LIF) - dependent signalling, a signalling pathway that is crucially important for the early stages of embryo development and for establishment of crosstalk between the embryo and the endometrium [89]. LIF is normally expressed in mammalian endometrium, with peak levels around the time of implantation. Endometrial deficiency of LIF in the secretory phase is commonly observed in women with idiopathic infertility and/or women with recurrent IVF failures [90]. The expression of LIF is directly regulated by p53 and carriership of variant alleles of the *TP53* gene has been shown to modulate the levels of LIF. The collection of experimental data for the effect of p53 on LIF production in humans is limited by ethical considerations, but carriership of the *TP53* P72 allele in transgenic mice and in human cell cultures was shown to be associated with at least 2-fold reduction in the expression of LIF than carriership of the Arg72 allele (Kang et al., 2009).

At least two polymorphisms in the LIF gene (the rs929271 SNP in the 3'-untranslated region and the Val64Met polymorphism have been shown to be overrepresented in young (< 35 years) women with idiopathic infertility and in sub-fertile young women (signified by a history of fertility treatments other than IVF) [43, 91, 92]. At the same time, carriership of the variant allele of the rs929271 *LIF* polymorphism was reported to be associated with increased ongoing pregnancy rates after IVF [93]. Apparently, carriership of this polymorphic allele may be associated with adverse effects on female fertility in young age and a beneficial effect at later age and/or after IVF, that is, it may represent an example of antagonistic pleiotropy [94].

Maternal as well as foetal homozygous genotype by the variant (G) allele of MDM2 SNP309 polymorphism (associated with increased level of MDM2, a negative regulator of p53) is specifically implicated in modulation of the risk for missed abortion [95].

Carriership of the *MTHFR* 677T allele confers increased risk for venous thromboembolism and vascular disease, especially in homozygotes [78], therefore, it is routinely included in the testing panel for inherited thrombophilia. Carriership of common pro-thrombotic mutations (Factor V Leiden, PTG20210A, MTHFR C677T) is known to increase the risk for pregnancy loss, but since the T allele is very common, the prevalence rates of homozygous genotypes are high (up to 30 % in some populations) [96] and co-inheritance with other, more rare prothrombotic mutations is not uncommon. Isolated 677T/T MTHFR genotype and its combination with other genetic prothrombotic factors is associated with significantly increased risk for pregnancy loss [97-99]. A similar effect on the risk for recurrent miscarriage has been identified for two other pro-thrombotic polymorphisms in the MTHFR gene, namely, the 1298A>C and the 1793G>A polymorphisms [97, 100]. It could be expected that this risk would be more pronounced in older pregnant women because of the increased likelihood for pre-existing or gestation-induced states that increase the risk for thrombosis (hyperglycemia, hyperlipidemia, hypertension). Nevertheless, the majority of the studies indicating for the association of carriership of prothrombotic mutations with increased risk for pregnancy loss were conducted in cohorts of younger carriers, therefore, genetic background and pre-existing or pregnancy-induced risk factors may be more important than age itself.

Carriership of single alleles associated with increased risk for infertility may not have a significant effect on the phenotype. Nevertheless, since most of these polymorphisms are quite common, carriership of more than one variant allele/s of genes coding for proteins with roles in DNA damage detection and repair and maintenance of genomic integrity may co-occur in the same woman, modulating the effect of each of the separate polymorphisms. For example, maternal homozygocity by the variant allele of the *MDM2* SNP309 polymorphism (associated with increased rates of missed abortion) co-inherited together with the rs17506395 in the *TP63* gene,

coding for a another member of the p53 family of DNA-binding proteins resulted in augmentation of the risk for recurrent pregnancy loss conferred by either of these polymorphisms [101]. Sometimes, the co-carriership of the polymorphisms in DNA repair genes may result in unexpected effects on the risk conferred by one or more of the separate polymorphisms. For example, in the study of Fraga et al. (2014), a combination of *TP53* 72Arg/Arg and *MDM2* SNP309 TT genotypes was shown to increase the risk for recurrent pregnancy loss [102]. Neither of the two polymorphisms, taken separately, was implicated the pathogenesis of pregnancy loss. At the same time, both the 72Arg allele of the *TP53* Pro72Arg and the T allele of the *MDM2* SNP309 polymorphism are associated with more pronounced 'pro-apoptotic' propensity. It is now believed that one of the complex causes for pregnancy loss is disruption of the balance between proliferation and apoptosis in the developing embryo. Thus, it is possible that the concurrent effects of the two polymorphisms in their homozygous state may increase the risk for apoptotic death of the embryo in the presence of even very low levels of DNA damage.

7. Role of the individual repair capacity in the constitution of the risk for pregnancy-induced or pregnancy-associated diseases and conditions

The prevalence of pregnancy-induced hypertension (PIH) is estimated to be 5-9 % and of prevalence of preeclampsia - 5-7 % of pregnant women, with the risk for first pregnancy being 4-5 times higher than for subsequent pregnancies, except in women where the interval between births was longer than 5-7 years [103]. It has long been suspected that oxidative stress and the resultant tissue damage formed the basis of the pathogenetic mechanisms of pregnancy-induced hypertensive disorders [104,105]. Oxidative metabolism is increased during pregnancy in order to fit the increased energy demands of the mother and the growing foetus. Normally, the increase in the levels of oxidative stress induced by pregnancy is promptly managed. It has been proposed that tissue damage secondary to unmanaged placental oxidative stress occurs when intrinsic mechanisms for management of oxidative stress cannot cope with the rates of placental production of ROS [106]. Increased levels of oxidative stress measured early in pregnancy (from 16 to 20 w.g.) have been shown to reliably predict the development of preeclampsia later [107]. Elevated levels of products of lipid peroxidation, 8-hydroxydeoxyguanosine (8-OH-dG), phosphorylated H2AX (both markers for the presence of DNA damage) and intermediates signifying ongoing DNA repair of oxidative damage have been repeatedly demonstrated in placentas from pregnancies complicated by preeclampsia compared to placentas from normal pregnancies [104-106, 108].

The first studies dedicated to the role of specific components of DNA repair pathways in the pathogenesis of complications of pregnancy such as preeclampsia and haemolysis/elevated liver enzymes/low platelets (HELLP) syndrome came from retrospective studies in families with children affected with the rare genetic disorder of trichothiodystrophy (TTD) type 1. TTD-1 is characterised by brittle sulphur-deficient hair, photosensitivity, developmental abnormalities and reduced life span [109]. Pregnancies with a TTD-affected foetus were retrospectively shown to have had abnormally high rates of pregnancy complications (placentation abnormalities, HELLP syndrome, preeclampsia), abnormal levels of routine biochemical markers, a significant percentage (70 %) of low birth weight infants (half of which were below the 10th percentile for gestational age) and were significantly more likely to have been delivered prematurely by emergency caesarean section because of fetal distress [110]. TTD-1 is caused by mutations in the gene *XPD* (*ERCC2*), coding for a DNA helicase unwinding the DNA at the site of damage and allowing unimpeded access of the cellular machinery for nucleotide excision repair (NER) [109]. *XPD* and the closely related gene *XPB* (coding for another helicase of NER) have been found to be expressed at very high levels in human placenta (highest among the levels of expression in any other human organ) in the course of normal pregnancy [111], starting from week of pregnancy 14 up to week 40, that is, widely encompassing the time of onset of early- and late-onset preeclampsia. Apparently, severely deficient foetal IRC was related to pregnancy complications associated with placental pathology. Considering that the placenta is a fetomaternal organ, the role of the maternal IRC in gestation-induced disorders associated with abnormal placentation became object of intensive study. So far, no association has been found between maternal carriership of the common *XPD* polymorphism *XPD* Lys751Gln (rs1052559) and the risk for preeclampsia [112, 113]. The results of the association studies have been controversial for the common polymorphisms Arg194Trp (rs1799782) and Arg399Gln (rs25487) in the gene coding for the protein XRCC1, a stabilising factor for the major ligase of base excision repair (BER, which is the main mechanism for repair of oxidative damage), with some studies reporting a statistically significant association between *XRCC1* Arg399Gln and preeclampsia [114] and other studies failing to observe an association [112, 113]. The MTHFR C677T polymorphism has also been implicated in the pathogenesis of preeclampsia [105], but the effects are

likely to be directly related to the hyperhomocysteinemia and the hypercoagulability associated with carriership of the T allele. The studies in the field are still very few and a potential age effect has not been examined so far. Hypertensive disorders of pregnancy are more likely to develop in women with pre-existing hypertension, obese women and women with diabetes than in women that were clinically healthy when they became pregnant. Therefore, the risks for hypertensive disorders of pregnancy are higher for older gravidas, but this is mainly related to the higher prevalence of risk factors in older women. The outcomes are usually dependent on the general health status of the woman and the quality of antenatal care they receive.

Gestational diabetes is a common complication of pregnancy, with prevalence varying between 5 % and 9 %, according to different sources [116]. It is associated with increased risk for adverse fetal and neonatal outcomes, including macrosomia, resulting in increased risk for birth trauma, neonatal hypoglycaemia and respiratory distress syndrome. Abnormal placentation and subsequent vascular dysfunction are common in women that later develop gestational diabetes. Poorly managed oxidative stress is known to be a major factor in the pathogenesis of insulin resistance and its associated complications [117-119]. Nevertheless, very few studies have been published with regard to the association of the maternal level of oxidative damage in and gestational diabetes, all of these in the last 5 years. In 2011, a pilot study revealed that the values of maternal urinary 8-OH-dG before 20 w.g. were strongly associated with the risk for gestational diabetes later [120]. This association was influenced by phenotypic factors, namely, maternal BMI, with overweight women with highest concentrations of urinary 8-OH-dG being at highest (> 5-fold) elevated risk for gestational diabetes. A later study of the levels of oxidised nucleotides (as a measure of DNA damage) in blood and urine of pregnant women demonstrated that there were differences in the DNA damage profile in pregnant women with mild pregnancy-induced hyperglycemia (normal results from oral glucose tolerance test but abnormal glycemic profile) and women with overt gestation diabetes (abnormal results from oral glucose tolerance test and abnormal glycemic profile) [121]. Specifically, both groups of patients presented with increased levels of oxidised bases in DNA, but those with mild pregnancy-induced hyperglycemia exhibited oxidative damage predominantly on pyrimidine bases whereas in those with gestational diabetes the damage profile included predominantly oxidation of purine bases in DNA. The authors of the cited study stated that the cause/s for the observed differences were yet unclear. It is possible; however, that the degree of impairment of the DNA repair systems (a result of a subtly lowered IRC and/or resulting from increased levels of oxidative stress) may explain the observed phenomenon. Oxidised pyrimidine bases are very commonly observed in DNA. The cytosine-derived products uracil glycol and 5-hydroxyuracil tend to mispair with adenine, resulting in a substitution of a C-G with an A-T pair upon next replication cycle. Nevertheless, the risks for nucleotide misincorporation in the course of replication of templates containing against oxidised cytosine derivatives depend on the sequence context with the correct nucleotide (guanine) being inserted most of the time, and only rarely C or A (resulting in a transition or transversion, respectively) [122]. Therefore, pyrimidine oxidation products are quite common, even under physiological conditions, and the presence of an oxidised pyrimidine in DNA is not very likely to result in a mismatch. At the same time, oxidised purine bases have a pronounced propensity to mispairing and are therefore considered highly mutagenic. 8-oxoguanine is likely to mispair with adenine in the course of normal replication, whereas 8-oxoadenine may mispair with C, creating a mismatch [123, 124]. There are designated mechanisms countering the potential mutagenic effects of purine oxidation, including specific enzymatic activities. It could be speculated that in a situation where the levels of oxidative stress are persistently elevated (but it is still managed), as in normal pregnancy, the residual level of unrepaired damage would be relatively low. In pregnancies where the oxidative damage is, for some reason, managed less efficiently (as in those with mild pregnancy-induced hyperglycemia), it could be expected that the profile of the residual unrepaired damage would comprise mainly products of pyrimidine oxidation, as these are very common anyway. In gestational diabetes, however, the level of oxidative damage is very high and the mechanisms that normally keep it in check are failing to keep pace. Thus, the profile of unrepaired oxidative damage is likely to contain all types of lesions, including the highly mutagenic purine oxidation products that are normally promptly dealt with.

Carriership of polymorphic variants of genes conferring decreased activity of key enzymes functioning in repair of oxidation damage (specifically, NEIL1, one of the three mammalian homologues of the bacterial BER glycosylase nei) have already been implicated in the pathogenesis of metabolic syndrome and diabetes type 2 in humans [119, 125]. It is possible that individual variance in the maternal capacity to recognise and repair oxidative damage may constitute a determining factor of the proneness to pregnancy-induced hyperglycemia. In any case, it could be expected that the impact of a subtle deficiency in the capacity to repair oxidative damage would be more pronounced in older rather than in younger pregnant

women, as the efficiency of the mechanisms for recognition and repair of damage tends to decline over time. In women with near-normal or superior capacity for DNA repair, however, it could be expected that the rates of pregnancy-induced diabetes would compare to the rates seen in younger women.

8. Role of the individual repair capacity in the constitution of the risk for prematurity and low birthweight infants

There is a small but representative number of studies linking the level of DNA damage (specifically, oxidative damage) and the risk for prematurity/low birthweight. Concomitant elevation in the levels of markers for DNA damage and markers for lipid peroxidation is indicative of mitochondrial damage as a result of unmanaged oxidative stress (because of high levels of generation of ROS due to inefficient energy utilisation and/or defective repair of oxidative damage). Elevated levels of urinary excretion of 8-OH-dG and malondialdehyde as a marker of lipid peroxidation were consistently observed in women that later gave birth to preterm infants, whereas lower levels of both metabolites were correlated with increased chances for term birth and normal birthweight [126]. Similar results were obtained in studies on maternal levels of 8-OH-dG and isoprostanes as a marker for lipid peroxidation, measured early in pregnancy [127]. The cited study also indicated that increased levels of isoprostanes alone were associated with increased risk for preeclampsia and that lower-than-normal levels of urinary 8-OH-dG were associated with increased risk for birth defects (probably indicating for foetal hypoxia and abnormal foetal circulation).

p53 and the p53-regulated pathways may they also play a role at later stages of pregnancy, besides their roles at pre-conception and implantation stages. Again, ethical considerations limit the amount of data for human reproductive biology, but studies carried out in female mice show that intrauterine deficiency of p53 is characterised by premature terminal differentiation and senescence-associated growth restriction of decidual cells, resulting in a significant increase in preterm births [128]. It is possible that carriership of known or yet unknown polymorphic variants of *TP53*, its transactivation targets and its regulators, resulting in subtly decreased activity or otherwise altered function of the p53 protein may affect successful carrying of human pregnancy to term as well. The number of studies directly associating carriership of variant alleles of genes coding for proteins functioning in DNA repair and maintenance of genomic integrity and risk for prematurity/low birthweight is, at present, very low. Maternal carriership of a polymorphism in the *TP53* gene, (namely, rs8079544 in intron 1), was recently found to play a role in the constitution of the risk for delivering preterm and low birthweight singleton infants [129]. The foetal genotype may also play a role. A retrospective study from 2011 demonstrated that shown that foetal carriership of the 72Arg allele of the *TP53* Pro72Arg polymorphism was associated with lower weight at birth [130]. Considering the pro-apoptotic properties of the 72Arg allele, it is possible that the increased rates of attrition of cells that have sustained damage in the course on intrauterine development result in smaller number of tissue precursors and, respectively, in lower body mass than in foetuses with Pro/Pro genotype.

The risks for pregnancy-induced complications, prematurity and low birthweight seem to be determined by the levels of extra oxidative damage generated in the course of pregnancy and the capacity of the mother and the growing foetus to manage damage efficiently. The degree of tissue damage and, respectively, the pregnancy outcomes may be very different in women with near-normal or superior capacity for repair of DNA damage and in women with inherited subtle deficiencies of DNA repair. These differences may be very small in younger women but may become prominent in older women, and, specifically, in those with pre-existing conditions and diseases associated with increased maternal levels of oxidative damage.

9. Conclusions

Pregnant women aged ≥ 35 may need to wait longer to conceive, are more likely to need assistance in conceiving, may experience higher rates of embryonic and foetal loss, may be at increased risk for chromosomal disease in the foetus, may exhibit higher rates of common pregnancy complications such as PIH and gestational diabetes and are more likely to give birth to premature and/or low birthweight infants than younger women. Nevertheless, the outcomes of pregnancies in women >35 depend on the pre-pregnancy health status and the quality of antenatal care the women receive, and may not be dramatically different from pregnancy outcomes in younger women. At present, data about the role of individual capacity for identification and repair of damage in DNA in the constitution of female fertility and fecundity is rapidly accumulating, indicating for a need for a more complex approach in the obstetrical management of older pregnant women.

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